

GROUND PORK STUDIES: QUALITY PARAMETERS AS AFFECTED BY  
DIFFERENT PACKAGING TREATMENTS AND THAWING METHODS;  
PROXIMATE ANALYSIS USING NEAR-INFRARED REFLECTANCE  
SPECTROSCOPY

by

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**Part I. Effect of packaging treatments and thawing methods  
on color, water-holding capacity, moisture content,  
protein, and drip losses of frozen ground pork.**

### Abstract

The effects of various packaging treatments and thawing methods on the quality of frozen ground pork stored for 16 weeks at -18°C were investigated. Color (Hunter L-value, a-value, b-value, %R630 - %R580 nm), water-holding capacity, moisture content, meat and drip protein, and drip losses were measured. Color as measured by %R630 - %R580 nm, a-value, and b-value was affected by both packaging treatments and thawing methods. Thawing at ambient temperature produced drip losses and resulted in samples with lower moisture content. Among the packaging treatments, aluminum foil produced the largest volume of drip when the pork was thawed at ambient temperature. However, among the inexpensive packaging treatments used during the study, aluminum foil offered the best protection against quality loss of frozen ground pork.

## Introduction

Freezer storage of meats has become an important method used by industry and consumers to preserve the quality of meats. Freezing of meats would not only increase the microbial safety of meat, but would also provide ample storage life to allow meat to be packaged and distributed to retail stores over long distances and stored at the place of consumption (Khan and Lentz, 1977). The freezing of meats raises problems which are related closely to its ultimate quality as a food and as judged by consumers. These problems may be identified as an increase in the loss of fluids (drip) during thawing, a decrease in juiciness due to this loss, and other changes in texture due to shortening and denaturation of proteins (Voyle, 1974).

During frozen storage, chemical and physical changes may occur. These changes include the development of oxidative rancidity, moisture loss, pH, and color changes often associated with freezer burn or oxidation. During freezing, ice crystallization and ionic shifts of minerals may result in loss of muscle protein solubility, causing a decrease in water-holding capacity and increase in drip (Kuo and Ockerman, 1984). Water-binding capacity of meat is higher with quick freezing at -55 °C than with slow freezing at -15 °C (Penny, 1974).

Oxidation of the color pigments in frozen meat systems

is of major concern because of the influence it can have on consumer acceptance. Color is affected by definite changes of the pigment myoglobin (Brooks, 1929). Immediately after a cut surface is exposed to air, the meat surface will become increasingly red in color; this form of the pigment is known as oxymyoglobin. The pigment eventually will oxidize to metmyoglobin which is brown or gray in color (Greene, 1971).

There is a great deal of confusion in the literature over the proportion of total drip which can be attributed to freezing and also over the effect of the rate of freezing on drip (Penny, 1974). Water which has been frozen is released and has to re-establish equilibrium with the muscle proteins and salt. Obviously, if the muscle proteins have been denatured, they will re-absorb less water resulting in loss of water soluble amino acids (Wladyka and Dawson, 1968). Bezanson (1975) suggested, however, that there are many serious potential causes of damage during thawing.

The packaging film also may play an important role in myoglobin oxidation (Seideman et al., 1983), bacterial count (Carpenter et al., 1975), evaporative losses, off-odors, and consumer acceptability (Rizvi, 1984).

Relatively oxygen-impermeable films, such as those used in vacuum packaging, usually will cause metmyoglobin

formation initially due to low oxygen tensions; but metmyoglobin will revert to reduced myoglobin when a substantial amount of the oxygen has been utilized and converted to carbon dioxide ( Seideman et al., 1983). Packaging material is used as a protective device for meat in frozen storage. Without a suitable packaging material, oxidative rancidity, moisture loss, and changes in color may occur. A moisture-proof freezer wrap is often used and acts as a barrier between the meat and the freezer environment.

Little work has been done on the effectiveness of recently-developed packaging treatments used by consumers in protecting meat during frozen storage. Therefore, the objectives of this study were: 1) to study the effectiveness of selected packaging treatments on the quality of ground pork by measuring water-holding capacity, drip losses, moisture content, protein, and color, 2) to evaluate the relationship between water-holding capacity, drip losses, and moisture content, and 3) to study the effect of packaging treatments and thawing method on drip losses.

## Literature Review

### Color

Color is an important quality consideration to the purchaser of meat in a retail market. Consumers consider the bright red color (oxymyoglobin) to be the desired color, especially for beef cuts and ground beef, and consider lack of this color to be a sign of product deterioration (Kropf et al., 1986; MacDougall, 1974). The primary cause of discoloration of frozen meat is the oxidation of oxymyoglobin to metmyoglobin (Seideman et al., 1983). The major factors that affect fresh meat color stability are temperature, gaseous environment in the package, oxygen consumption, and the reducing capacity of the meat (MacDougall, 1982). Gaseous environment, oxygen consumption, and the reducing capacity of the meat are all affected by temperature. Pork has the lowest concentration of myoglobin such that it is the lightest in color when compared to other red meats (Walters, 1975). Low pH will cause the muscle fibrils to be more open and scatter light and, thus, appear paler in color (Seideman et al., 1983).

Color of meat and meat products is dependent on the concentration of meat pigments, the chemical state of the pigment, and the physical characteristics of the meat such as the light scattering properties (Kropf et al., 1986; Greene, 1971; Seideman et al., 1983). The most common

chemical states for myoglobin are deoxymyoglobin, commonly referred to as reduced myoglobin; oxymyoglobin and metmyoglobin. Deoxymyoglobin has iron in the ferrous state and is dark red to purplish-red in color. It occurs at extremely low oxygen partial pressures. Oxymyoglobin is a bright red pigment which forms very quickly after deoxymyoglobin is exposed to oxygen. The iron must be in the ferrous state for oxygenation to occur (Livingston and Brown, 1981). The oxidized form of myoglobin has iron in the ferric state and is known as metmyoglobin. Metmyoglobin has an undesirable brown color which the consumer associates with off-conditioned meat. In fresh meat, pigment oxidation to metmyoglobin is affected principally by the reducing capacity of the muscle, oxygen availability, and temperature (MacDougall, 1982). According to Greene (1969), consumers will reject beef containing over 40% metmyoglobin on the surface. MacDougall (1982) found a brown to gray-greenish color to be associated with 60% metmyoglobin, a distinctly brown color to be associated with 40% metmyoglobin, and consumer discrimination to begin at 20% metmyoglobin content. Robach and Costilow (1962) reported that aerobic bacteria contributed to metmyoglobin formation by reducing oxygen tension at the meat surface.

The interrelationship between color and flavor (between metmyoglobin and rancid flavor) becomes apparent

when ferric hemes (metmyoglobin in raw meat, denatured globin hemichrome in cooked meat) catalyze oxidation of polyunsaturated fatty acid (PUFA) (Greene, 1971). The causes of product rejection are appearance and aroma-flavor problems related to microbiologically-caused deterioration and by aroma-flavor problems resulting from fat oxidation (Kropf and Hunt, 1984).

Several methods are available to measure color. The method of pigment extraction and transmission or absorption spectrophotometry does not prevent the conversion of one myoglobin form to another and, therefore, provides no reliable information on pigment form stability, resulting in overestimation of metmyoglobin and oxymyoglobin and underestimation of deoxymyoglobin content (Dean and Ball, 1960). A problem with using extraction techniques to quantify pigment form proportion is that of what thickness of the muscle surface should be sampled relative to the pigment layers seen visually (Kropf et al., 1984). Reflectance spectrophotometry eliminates the need for extraction and allows the pigment to be evaluated in its natural environment (Franke and Solberg, 1971). A HunterLab Spectrophotometer may be used to measure muscle color changes or for estimation of myoglobin (Hunt and Kropf, 1985). The L-, a- and b-scales give measures of color in terms of approximate visual uniformity throughout the solid

color. L-scale measures lightness and varies from 100 for perfect white to 0 for black. The a-scale measures redness when positive, gray when zero, and greenness when negative. The b-scale measures yellowness when positive, gray when zero, and blueness when negative (Instruction manual, HunterLab, 1979). Reflectance differences between wavelengths 630 nm minus 580 nm or the ratio of 630 nm / 580 nm have been useful in experiments where redness differences exist or develop (Hunt and Kropf, 1985). Wavelength 580 nm is an absorption peak for oxymyoglobin, while wavelength 630 nm is an absorption peak for metmyoglobin and an absorption minimum for oxymyoglobin (Hunt, 1980).

#### Packaging Materials

In order to maintain the optimal quality of fresh meats during transit and subsequent storage, prevention of shrinkage, discoloration, and microbial growth is necessary (Holland, 1980). Rizvi (1984) found that of the many and varied roles of packaging materials, providing protection to foods against harmful environmental factors was considered the most important. To maintain product quality, protection from natural and man-made elements that increase deterioration is required. The films utilized in fresh meat packaging vary in physical properties which results in variations in oxygen permeability, moisture vapor

transmission rate, shrinkage properties, brittleness, and clarity (Carpenter et al., 1975). In vacuum packaging, CryoVac<sup>R</sup> bags were used with SuperVac<sup>R</sup> equipment. CryoVac<sup>R</sup> bags are made of a coextrude film of a mixture of ethylene vinyl acetate, polyvinyl, and polyvinylidene chloride copolymer. It has a water vapor permeability of 7.2 g/m<sup>2</sup> per day, at 30°C and relative humidity of 78%. Its oxygen transmission rate is 37.5 cm<sup>3</sup>/m<sup>2</sup> per one atmosphere per day at 25°C and a relative humidity of 75%. Saran Wrap<sup>R</sup> is a polyvinyl and polyvinylidene chloride copolymer. CryoVac<sup>R</sup> bags, Saran Wrap<sup>R</sup>, and polyvinylchloride are heat shrinkable materials. Shrinking reduces the oxygen transmission rate approximately in proportion to the reduction in area (Zarate and Zaritzky, 1985). Oxygen transmission rate of the composite film decreases at low temperatures following the Arrhenius relationship (Eustace, 1981). Previous studies suggested the following implications on film packaging of meats: a) shrinkage and surface desiccation are drastically reduced by covering fresh meat with films of low moisture vapor transmission, b) cuts which are vacuum packaged can be stored for long periods of time (6 - 12 weeks) and retain the ability to become bright red when placed under aerobic conditions. After two weeks of storage at 3.3 °C, anaerobically packaged beef was found to be similar in taste and quality

to fresh beef, whereas aerobically packaged beef became unacceptable in 1 week (Rizvi, 1984). Shrinkage losses for lamb cuts wrapped in polyvinylchloride (PVC) film were substantially reduced, and the fat surface was extremely attractive (Smith et al. 1974; Berry et al. 1971). Unfortunately, the use of PVC film was associated with a substantial increase in bacterial count.

Various researchers reporting on various systems for storage of pork cuts concluded that vacuum packaged loins sustained less shrinkage than loins wrapped in paper, had less surface discoloration than loins wrapped in PVC and parchment paper, had lower incidence of off-odor, and were higher in consumer acceptability than loins wrapped in PVC. Film packaging of meat can successfully prevent evaporative losses and desiccation of fresh meat surfaces (Smith et al., 1974; Terlizzi, 1982; Anjaneyula and Smidt, 1986).

Use of films which are permeable to oxygen will allow growth of aerobic psychrotrophic bacteria which frequently contribute to off-odors, discoloration, and ultimate spoilage (Carpenter et al., 1975; Rizvi, 1984; Terlizzi, 1982). Sebranek (1986) suggested that use of water-impermeable film will provide essentially 100 % relative humidity within the package, thus minimizing shrinkage and providing assistance to color retention. The storage life of vacuum packaged beef was found to be inversely related

to film permeability to oxygen (Newton and Rigg, 1979). This relationship was found for both quality (color, odor) and microbial count.

Jeremiah (1980) evaluated the effects of frozen storage in various protective wraps upon the cooking losses and palatability of various fresh and cured pork cuts. Flavor and overall palatability of both fresh and cured cuts decreased during frozen storage at -30 °C up to 196 days. The deterioration was attributed to the development of oxidative rancidity. There were no significant differences among protective wraps evaluated for any of the traits measured. Ashby and James (1974) studying the effects of packaging on freezer burn found no significant differences in packaging methods.

#### Thawing Method

Thawing involves melting all of the ice crystals in frozen products. As thawing proceeds into a meat block, the thawed outer layer limits heat flow to the inside, so it takes longer to thaw than it does to freeze under the same conditions of heat transfer (Bezanson, 1975). During thawing, the surface of the meat is exposed to bacterial growth and other deteriorative changes throughout the time that the center is thawing. Marriott et al. (1980) suggest a need to thaw ground beef at refrigerator temperature to reduce public health concern. Gonzalez-Sanguinetti et al.

(1985) concluded that thawing involved the reabsorption of water by the fibers, and the exudate produced was at least part of the water not reabsorbed by the fibers. Water which has been frozen is released and has to re-establish equilibrium with the muscle protein and salts. Obviously if the muscle proteins have been denatured they will reabsorb less water. Furthermore, since the fibers have been squeezed and distorted by ice formation, this non-reabsorbed water will lie in wider channels within the meat structure, and, thus, will increase the potential of drip formation (Penny, 1974).

The amount of water which may exude from meat as drip after freezing and thawing depends on a number of factors of which the ratio of cut surface to weight or volume is the most important (Zarate and Zaritzsky, 1985; Khan and Lentz, 1977). It is clear that the free water has to move to the surface before it can drip from the meat and, therefore, the more cut surface to volume, the less distance the water has to travel (Anon and Calvelo, 1980). The drip also is reduced if the pieces are cut along the direction of the fibers rather than across them. The amount of drip which is found depends on the conditions of the meat as a result of its post-mortem treatment; pH, size of meat when it was frozen, conditions of freezing, temperature and time of storage, size of the pieces of meat

when thawed, and conditions of thawing (Zarate and Zaritzky, 1985; Penny, 1974; Anon and Calvelo, 1980). Gonzalez-Sanguinetti et al. (1985) studied the effect of thawing rate on exudate production of frozen beef and concluded that: 1) thawing conditions affected the amount of exudate by giving more or less time for extra-cellular water reabsorption, and the reduction in water-holding capacity would only depend on freezing and storage conditions, 2) the amount of exudate became independent of the thawing rate when the reabsorption period was long enough, and 3) histological experiences showed that non-reabsorbed water was accumulated in the extra-cellular space suggesting that the main resistance to water reabsorption by the fibers was imposed by water migration through the sarcolemma. Anon and Calvelo (1980) indicated that if the fibers had been distorted and/or the proteins altered by the increase of ionic force (possible effects of slow freezing), less water would be reabsorbed, thus increasing exudate production. Also, if the cell wall had been damaged by freezing, the amount of exudate released was even greater. They further stated that although total concentration of proteins in the exudate was higher for unfrozen samples than that for frozen samples, statistical analysis failed to show that those differences were significant.

The concentration of amino acids in both dark and light meat decreased and the concentration in the drip increased with increasing storage time (Wladyka and Dawson, 1968). In addition, larger quantities of essential amino acids were detected in drip from frozen light meat compared to dark meat after both periods of storage. Bezanson (1975) found that drip or blood loss had a protein content of about 10 %. Awad et al. (1968) concluded that the protein content in thaw drip was much higher than that in cook drip for each storage time. Since amino acids, peptides, and inosinic acid are known to impart and enhance the flavor of meat (Mabrouk et al., 1969; Wasserman and Gray, 1965), the increased loss of this materials in drip may appreciably affect the flavor of a slowly-frozen product or meat.

#### Water-holding Capacity

The ability of meat to retain its natural water content (water-holding capacity) has been extensively reviewed (Offer and Trinick, 1983). In general, it is accepted that freezing produces some changes in the tissue which reduce the water-holding capacity after thawing. Ice formation raises the solute concentration in the tissue which, in turn, may induce denaturation of proteins, disturbance of the existing equilibrium between structural water and protein, and alteration of the membrane permeability (Gonzalez-Sanguinetti et al., 1985). Fennema

(1973) stated that the loss of water-holding capacity in beef muscle, especially myofibrillar proteins, as a consequence of protein denaturation is a well-known fact for long storage times. Awad et al.(1968) found that the water-holding capacity of bovine muscle was reduced considerably during 8-week storage at -4 °C. Water-holding capacity may vary among muscles originating from animals of the same species, breed, sex, weight, age, and ante- and post-mortem treatment, and affects both quality and composition.

Kauffman et al. (1986) stated that muscles releasing excess fluids are dryer tasting and lose more weight during processing, storage, transit, and display, thus altering composition, salable weight, and visual appearance. At pH levels considerably above ( $> 6.0$ ) or below ( $< 4.0$ ) the iso-electric point (approximately 5.0), the number of available charges is enhanced, thus increasing water-holding capacity (Gault, 1985; Bouton et al. 1972). Gault (1985) also found that increased water-holding capacity, as measured by swelling ratio in both raw and cooked meat, markedly influenced cooked meat tenderness irrespective of the connective tissue content of the muscle. Khan and Lentz (1977) found that water-holding capacity decreased with increasing storage time and temperature. They further stated that loss of water-holding capacity was indicated by

higher drip and cooking losses.

Bouton et al. (1972) stated that the differences found in water-holding capacity were not significant when the moisture lost in weep during storage was taken into account. The current literature is not conclusive as to whether different packaging treatments affect water-holding capacity. Although ice crystallization and ionic shifts of minerals may result in loss of muscle protein solubility, causing a decrease in water-holding capacity and increase in drip, water holding capacity of meat was found to be less affected by quick freezing than by slow freezing. Water-holding capacity was found to be affected by fat content in spite of the fact that the percentage of moisture was used in the calculation. Tsai and Ockerman (1981) found that samples with very high fat content were very high in water-holding capacity by the press method and very low in water-holding capacity by the centrifugal method. For meats with high fat content, greater area of meat film will be observed because of the mixture of fat and meat in the film. Pork with a higher fat content will give three areas: a water area, a fat area, and a bound tissue area which makes it difficult to differentiate between the fat area and the bound area because of low pigmentation when using the press method (Tsai and Ockerman, 1981).

#### Moisture Content

Packaging materials also have an effect on the total moisture content of the protected material. Baldwin et al.(1972) found wax paper to be less effective against water loss than were moisture-vapor-proof wraps. Cooper (1970) reported that unwrapped bacon sides shrank 3.1 % after 2 months storage, while bacon sides wrapped in polyethylene lost no weight. He concluded that a moisture-vapor-proof wrap appeared to be essential to prevent significant weight loss in meats.

## Materials and Methods

### Materials

Pork was obtained from a commercial source (Flint Hills Foods, Alma, Kansas) and ground with a Hobart grinder (Model 4732) using 3/16" and 1/8" plates. The fat content of duplicate samples was estimated with a Hobart fat tester (model F-101). Immediately after grinding, the ground pork samples were analyzed for color, water-holding capacity, protein, fat, and moisture. Before freezing, the ground pork was packaged in various wraps in 0.57 kg. (1.4 lb.) quantities. The packaging treatments included vacuum packaging, Saran Wrap<sup>R</sup> (polyvinyl and polyvinylidene chloride copolymer), Reynolds heavy duty aluminum foil<sup>R</sup>, Saran Wrap<sup>R</sup> overwrapped with aluminum foil<sup>R</sup>, and PVC (polyvinylchloride). In vacuum packaging, CryoVac<sup>R</sup> bags (made of a coextruded film of a mixture of ethylene vinyl acetate, polyvinyl and polyvinylidene chloride copolymer) were used with SuperVac<sup>R</sup> equipment. The CryoVac<sup>R</sup> bags, Saran Wrap<sup>R</sup>, and PVC are heat shrinkable materials. The packaged samples were held at -18 °C for 16 weeks. Each of 4 replications was placed in an upright Hotpoint freezer at 1-week intervals. After the designated storage time, the samples were thawed by one of 2 methods, at ambient temperature (approximately 25°C) for 4 hours, or thawing at 4 °C for 8 hours on the bottom shelf of the refrigerator.

During both thawing treatments, samples were placed on broiler pans and loosely covered with Saran Wrap<sup>R</sup>. Samples were considered thawed when the internal temperature was 4°C. Drip was collected and measured using a graduated cylinder. The thawed samples were analyzed separately as outlined below.

#### Color

Color was measured with a Hunter D54 Lab spectrophotometer using Illuminant A (Hunt and Kropf, 1985). The spectral reflectance was read at 10 nm intervals, and values of L, a, b were taken in duplicate for each sample. Each sample was rotated 180° and read again. The equation, %R 630nm - %R 580nm, was used to estimate the bright red color of oxymyoglobin.

#### Total Protein Nitrogen

The total protein nitrogen content for each sample was determined by Buchi-Semi-Micro Kjeldahl procedure (AOAC, 1984). The protein content was calculated by multiplying the nitrogen content by a factor of 6.25.

#### Moisture

Five gram of each sample was weighed before drying in moisture tins (aluminum), and weighed after drying for 16 hours at 105 °C (AOAC, 1984). Total moisture content was determined by weight loss.

#### Fat Content

Fat was extracted from the samples by the Soxhlet method using petroleum ether (AOAC, 1984). Percent fat was determined by weight loss using the following equation: (dry weight before extraction - dry weight after extraction)/dry weight before extraction and multiplied by 100.

#### Water-holding Capacity

Water-holding capacity was determined by the Hamm Press method (Hamm, 1963) and calculated using the following equation:

$$W H C = 1 - E L I \text{ where,}$$

$$E L I = (\text{area of expressed juice} / \text{area of pressed meat}) \times 100$$

#### Thaw Drip

The volume of drip from each sample was measured using a graduated cylinder. The drip was also analyzed for total protein nitrogen content using the Buchi-Semi-Macro Kjeldahl method (AOAC, 1984), and the protein content of the drip was obtained by multiplying its nitrogen content by 6.25.

#### Statistical Procedure

Data were analyzed with the Statistical Analysis System (SAS) as a 5 X 2 factorial randomized complete block design with four replications (batches of meat). All effects in the model were considered fixed. The Fisher's

Protected Least significant difference (LSD) test for mean separation was used to test the significance of differences between treatment means (Ott, 1984).

## Results and Discussion

### Packaging Treatments

The packaging treatments utilized during the study are presented in Table I-1. PVC was considered as highly oxygen-permeable and vacuum as oxygen-impermeable. Saran Wrap<sup>R</sup> is relatively oxygen impermeable; and it keeps in moisture and aroma, retains product freshness, keeps out drying air and prevents dehydration.

The mean squares and F-values for packaging treatments for the objective parameters [moisture, water-holding capacity, color (L-, a- and b-values, and %R630-%R580), meat protein, drip protein, and drip loss] of frozen ground pork are presented in Table I-2. There was no significant difference among packaging treatments for moisture, water-holding capacity, L-value (lightness), meat protein, and drip loss of frozen ground pork after 16 weeks of storage. The lack of statistical significance for moisture content of frozen ground pork stored on various packaging treatments is in agreement with the findings of Clark (1985) and Cooper (1970). On the other hand, Ashby et al. (1973 a, b) found that shrinkage of ham was dependent mainly on dehydration during initial freezing, reabsorption of available moisture during storage and thawing, and storage time. Unwrapped bacon sides shrank 3.1% after 2 months of storage while wrapped bacon sides (polyethylene)

lost no weight (Ashby and James, 1974). Our results indicated that although the packaging treatments have different oxygen-permeability and water-vapor transmission rate, they were able to offer equal protection against moisture loss.

The non-significance of water-holding capacity of ground pork packaged in various packaging treatments in this study is in agreement with Bouton et al. (1972). This might be due to the high fat content of the meat. Tsai and Ockerman (1981) found that samples with high fat content were high in water-holding capacity by the press method. Awad et al., (1968) found that the protein content of drip calculated as a percentage of total muscle protein, increased from about 5.86 for unfrozen muscle to 17.03 for muscle frozen for 8-weeks. The greatest increase in the amount of protein in drip occurred during the first four weeks of storage; thereafter, the increase were comparatively insignificant. Sarcoplasmic protein content decreased from about 29% to 13% during frozen storage. The lack of significant variation for L-value from this study (Table I-3) agrees with MacDougall (1974), but is at variance with the findings of Anjaneyula and Smidt (1986) that pork chops became lighter in color with an increase in storage time. The color of frozen meat varies with the rate of freezing. Differences in frozen meat lightness

result from the dependence of ice crystal growth on the freezing rate. Small crystals formed by fast freezing scatter more light than large crystals formed by slow freezing, and hence, fast frozen meat is opaque and pale while slow frozen meat is translucent and dark. In addition to the rate of freezing, the duration of exposure to air before packaging and freezing is important. In thawed meat, the rate of pigment oxidation is increased. Therefore, the color will be less stable than in fresh meat. Since meat color is considered a surface phenomenon of a non-metallic opaque object, the extent to which meat appears glossy is related to the thin aqueous layer on the surface and muscle pH, water-holding capacity, structure, and fiber orientation (Hunt, 1980). Perhaps glossiness had some effect on the lightness value. Fresh lamb cuts were more desirable in color than freezer stored cuts regardless of packaging treatments (Carpenter et al., 1975).

There was significant variation among the various packaging treatments for a-value, b-value, %R<sub>630</sub> nm-%R<sub>580</sub> nm, and volume of drip loss of the frozen ground pork.

Vacuum packaged ground pork gave the highest value for redness (Hunter a-value) (Table I-3). This value was higher than meat packaged with PVC, aluminum foil, Saran Wrap<sup>R</sup> overwrapped with aluminum foil, or Saran Wrap<sup>R</sup>. Vacuum packaging affected the gaseous environment at the meat

surface. Oxygen, which remained in the package and in the meat after the bag had been evacuated and sealed was depleted by the metabolic activity of the meat itself and of the bacteria at the surface of the meat. This resulted in the formation of deoxymyoglobin, due to low oxygen tension. Deoxymyoglobin will revert to the bright red oxygenated pigment (oxymyoglobin) when exposed to oxygen, producing the familiar "bloom" of fresh meats. Anjaneyula and Smidt, (1986) also found that the intensity of redness remained more or less the same for meat samples packaged in high vacuum whereas other treatments showed decreased redness. PVC gave the next highest a-value. This result did not agree with that of Clark (1985) who found that aluminum foil showed the greatest protection of the red color of ground pork. Saran Wrap<sup>R</sup> gave the lowest protection to redness. Aluminum foil seemed to protect redness better than Saran Wrap<sup>R</sup> overwrapped with aluminum foil. Saran Wrap<sup>R</sup> appeared to offer less protection against the loss of the red color of the meat than did most of the other materials utilized. With regard to the physical and chemical characteristics of Saran Wrap<sup>R</sup> and PVC, one would expect Saran Wrap<sup>R</sup> to offer better protection against the loss of the red color, in other words, give a higher a-value than PVC. But this effect of freezing rate on gas environment in the package was not the case in our study.

Saran Wrap<sup>R</sup> has a low oxygen permeability rate and water vapor transmission rate, thus Saran Wrap<sup>R</sup> would minimize the loss of color, keep air out and moisture in, eliminating freezer burn and color loss due to moisture loss. There was a significant negative correlation ( $r=-0.40$ ) between the L-value and a-value as indicated in Appendix I-1. The lightness index (L-value) of beef was shown to be highly correlated with water loss and initial pigment concentration ( Lanier et al. 1977).

Of all packaging treatments, vacuum packaging resulted in a lower b-value for frozen ground pork (Table I-4). Even though not statistically significant PVC gave the highest value for yellowness. This could be attributed to the fact that PVC is oxygen-permeable, thus resulting in oxidation of the color pigments and loss of moisture which will affect the thin aqueous layer on the surface. The yellowness value of PVC was followed by Saran Wrap<sup>R</sup>, Saran Wrap<sup>R</sup> overwrapped with aluminum foil, aluminum foil, and vacuum packaging, respectively. These results are in agreement with results in Table I-3 where vacuum packaging gave the highest protection for redness of ground pork. Besides vacuum packaging, aluminum foil, although not statistically different from other treatments, seemed to offer the most inexpensive and better protection against the development of yellowness in meat during frozen

storage; and it offered the best protection for redness when compared to Saran Wrap<sup>R</sup> and Saran Wrap<sup>R</sup> overwrapped with aluminum foil as indicated in Table I-3.

Among the different packaging treatments, vacuum packaging gave the highest %R630-%R580 nm value (Table I-5), an indicator of a higher proportion of oxymyoglobin. This result agrees with that in Table I-3 where vacuum packaging gave the highest a-value. Vacuum packaging protects against the loss of redness in meat. This is important because consumers consider the lack of red color to be a sign of product deterioration. Saran Wrap<sup>R</sup> was the lowest. Again, aluminum foil and PVC seem to offer similar protection in preventing the loss of red color in meat. PVC was different ( $p \geq 0.05$ ) from Saran Wrap<sup>R</sup>, while Saran Wrap<sup>R</sup> overwrapped with aluminum foil and aluminum foil were not different from Saran Wrap<sup>R</sup> and PVC. Although they were not different, when considering inexpensive packaging treatments, it would seem that, since aluminum foil offers the best protection against color (redness, yellowness, and %R630-%R580), and Saran Wrap<sup>R</sup> has low oxygen permeability and water-vapor transmission rate, better overall quality protection would be enhanced if these materials (Saran Wrap<sup>R</sup> overwrapped with aluminum foil) are used. There was a positive correlation ( $r=0.79$ ) between a-value (redness) and the difference between

wavelengths (%R630-%R580 nm) (Appendix I-1).

Meat wrapped in aluminum foil gave the most thawing drip (Table I-6). When meat is tightly wrapped, this tends to distort it forcing out drip which is then trapped on the surface of the meat. Tight wrapping was found to increase drip loss by an average of 1.2% (Malton and James, 1983). Air should be eliminated from the package to prevent shrinkage and freezer burn; and since aluminum foil is strong, flexible, resistant to tearing, and molds easily to press out air pockets, a tendency to tightly wrap the meat due to the easy molding might result in more drip. Heat shrinking of the film reduces release of weep, and this is important when thawing at higher temperatures. PVC was not different ( $p > 0.05$ ) from Saran Wrap<sup>R</sup> and Saran Wrap<sup>R</sup> overwrapped with aluminum foil. Vacuum packaging seemed to offer the best protection against drip loss. This is important because drip loss was found to contain between 8% to 10 % protein (Bezanson, 1975). The protein content in thaw drip was found to be much higher than that in cooked drip (Awad et al. 1968). Since amino acids, peptides, and inosinic acid are known to impart and enhance the flavor of meat, the increased loss of these materials in drip may affect the flavor of the cooked meat. The nutritional quality of the product may be affected by the amount of drip produced during thawing. From the results presented in

Table I-6, one would discourage the use of aluminum foil as a freezer packaging treatment because of the amount of drip loss produced from thawing unless the wrapped meat is thawed at 5°C, or the drip is incorporated before of during cooking.

#### Thawing methods

There was no difference ( $p > 0.05$ ) between thawing methods for water-holding capacity, L-value, %R630-%R580 nm and drip protein % of the frozen ground pork (Table I-7). Significant variation between thawing methods was only found for moisture content, a-value, and b-value. Thawing at refrigerator temperature did not produce any drip, thus, excluding drip loss and drip protein from the analysis of variance for thawing methods. This can be explained by the fact that there is competition for water between the crystals of intracellular and extracellular growth. This avoids cellular distortion and the outlet of water to the extracellular space. When thawing has taken place, as the water has not been released from the fibers, it is reincorporated and retained more easily resulting in no drip loss. Movement of water (during thawing in the refrigerator) may have been diffusional and weep was produced by gravity forces (during thawing at ambient temperature). Thawing at low temperature (10°C) resulted in lower drip loss, firmer texture, and higher ratings for

juiciness and tenderness but took much longer than thawing at higher temperatures ( $21.1^{\circ}\text{C}$  (Footrakul, 1976). There was no significant packaging treatments  $\times$  thawing method interaction for any of the parameters evaluated in this study.

The effects of thawing methods on moisture, a-value, and b-value of frozen ground pork are presented in Table I-8. Thawing the frozen ground pork at refrigerator temperature gave a higher ( $p \leq 0.05$ ) a-value than did thawing at ambient temperature. This might be due to the amount of light in the room as compared to the amount of light in the refrigerator and the amount of evaporative losses during thawing which affect the amount of light reflected by the sample. Thawing meat covered with materials which preclude the exclusion of air from the package result in rapid moisture migration from the meat to the packaging material giving a snow-like appearance on the film and a brown dehydrated meat surface. Thawing at refrigerator temperature gave also a higher ( $p \leq 0.05$ ) b-value than thawing at ambient temperature.

Thawing in the refrigerator resulted in samples with higher ( $p \leq 0.05$ ) moisture content than samples thawed at ambient temperature. This might be due to the fact that there was no drip losses for samples thawed at the refrigerator temperature. The water was reabsorbed by the

fibers resulting in a product high in moisture content. Thawing at ambient temperature produced drip losses while samples thawed in the refrigerator temperature did not produce any drip, but thawing in the refrigerator took twice the amount of time as thawing at ambient temperature.

### Summary

Ground pork (0.57 kg. samples) with a fat content of 20 ± 4% was frozen and stored for 16 weeks in various packaging treatments [Saran Wrap<sup>R</sup>, Polyvinylchloride (PVC), Aluminum foil<sup>R</sup> (heavy duty), vacuum packaging, and Saran Wrap<sup>R</sup> overwrapped with Aluminum foil] and thawed at both ambient (25<sup>0</sup>C) and refrigerator (4<sup>0</sup>C) temperatures. During thawing, the samples were placed on broiler pans and covered with Saran Wrap<sup>R</sup> in order to collect the drip. After thawing, the samples were analyzed for moisture content, meat and drip protein, water-holding capacity, color (L-, a-, b-values, %R630 - %R580 nm), and drip losses. Packaging treatments and thawing methods effected quality of frozen ground pork.

Significant differences among packaging treatments for color (redness, yellowness, %R630 - %R580 nm) and drip loss were observed in this study. Vacuum packaging protected against redness, yellowness, %R630 - %R580 nm, and drip loss. Saran Wrap<sup>R</sup> offered the least protection for maintaining redness and %R630 - %R580 while PVC gave the highest value for yellowness. The packaging treatments had no effect on moisture content, water-holding capacity, color (L-value), meat and drip protein content.

Thawing at ambient temperature resulted in drip losses with Aluminum foil producing the largest volume of drip and

vacuum packaging and Saran Wrap<sup>R</sup> producing the smallest volume of drip. Moisture content and color (redness and yellowness) were affected by the thawing method. Thawing at refrigerator temperature ( $4^{\circ}\text{C}$ ) resulted in samples which were high in moisture content, redness and yellowness.

Water-holding capacity, drip loss, and moisture content were only weakly related in this study.

### Conclusions

Under the conditions of this study:

- 1) Packaging treatments and thawing methods appeared to have an effect on the quality of frozen ground pork.
- 2) Among the objective parameters studied, color (redness, yellowness, and %R630-%R580 nm) was found to be affected by both packaging treatments and thawing methods.
- 3) Thawing at ambient temperature produced drip losses and reduced product moisture content.
- 4) Among the packaging treatments aluminum foil produced the largest volume of drip while vacuum and Saran Wrap<sup>R</sup> produced the smallest volume of drip.
- 5) Pork wrapped with Saran Wrap<sup>R</sup> and overwrapped with aluminum foil had no advantage over other packaging treatments for the objective parameters evaluated in this study.
- 6) Water-holding capacity, drip loss, and moisture content were only weakly related.
- 7) For better quality retention during frozen storage of ground pork, vacuum packaging seems to offer the best protection followed by aluminum foil, Saran Wrap<sup>R</sup> and PVC.

Table I-1. Packaging treatments utilized.

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Aluminum foil, heavy duty <sup>Ra</sup>
Polyvinylchloride (PVC)
Saran Wrap <sup>Rb</sup>
Saran Wrap <sup>Rb</sup> + aluminum foil <sup>Ra</sup>
Vacuum <sup>Rc</sup>

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<sup>a</sup>Reynolds Chemical Co.

<sup>b</sup> Dow Chemical Co.

<sup>c</sup> CryoVac (7" x 12 " bags)

Table I-2. Mean<sup>1</sup> squares and F-values from analyses of variance for packaging treatments of ground pork stored for 16 weeks at -18°C.

Parameter	Mean square	F-value
% moisture	1.47	1.35
Water-holding capacity <sup>a</sup>	9.25	1.78
Color <sup>b</sup> :		
L-value (lightness)	1.91	1.61
a-value (redness)	6.51	6.31***
b-value (yellowness)	0.37	7.32***
% R630-%R580 nm	39.85	6.64***
Meat protein	0.38	1.13
Drip protein	0.26	0.42
Drip loss	31.69	20.63***

<sup>1</sup>Mean of 4 replications

<sup>a</sup>Measured by Hamm Press method

<sup>b</sup>Measured by HunterLab D54 reflectance spectrophotometer

\*\*\* p<0.001

Table I-3. Means<sup>1</sup> for L- and a-value of ground pork stored in different packaging treatments for 16 weeks at -18°C.

Packaging treatments	L-value	a-value
Saran Wrap <sup>R</sup>	52.65a	14.01b
Saran Wrap <sup>R</sup> + aluminum foil	53.22a	14.29b
Aluminum foil	52.91a	14.41b
PVC	52.97a	14.97b
Vacuum	51.95a	16.29a

<sup>1</sup> Mean of 4 replications

ab Means with the same letter within a column are not different ( $p > 0.05$ )

Table I-4. Means<sup>1</sup> for b-value of ground pork stored in different packaging treatments for 16 weeks at -18°C.

Packaging treatments	b-value
Vacuum	5.28b
Aluminum foil	5.65a
Saran Wrap <sup>R</sup> + aluminum foil	5.74a
Saran Wrap <sup>R</sup>	5.74a
PVC	5.83a

<sup>1</sup>Mean of 4 replications

abMeans with the same letter within a column are not different ( $p \geq 0.05$ )

Table I-5. Means<sup>1</sup> for the difference in wavelengths (%R630-%R580 nm) of ground pork stored in different packaging treatments for 16 weeks at -18°C.

Packaging treatments	%R630 -%R580
Saran Wrap <sup>R</sup>	12.88c
Saran Wrap <sup>R</sup> + aluminum foil	14.06bc
Aluminum foil	15.01bc
PVC	15.44b
Vacuum	18.83a

<sup>1</sup>Mean of 4 replications

abcMeans with the same letter within a column are not different ( $p \geq 0.05$ )

Table I-6. Means<sup>1</sup> for volume of drip (after thawing) of ground pork stored in different packaging treatments for 16 weeks at -18°C.

Packaging treatments	Volume (ml)
Vacuum	3.68d
Saran Wrap <sup>R</sup>	6.83c
PVC	7.63bc
Saran Wrap <sup>R</sup> + aluminum foil	9.03b
Aluminum foil	11.30a

<sup>1</sup>Mean of 4 replications

abcdMeans with the same letter within a column are not different ( $p \geq 0.05$ )

Table I-7. Mean<sup>1</sup> squares and F-values from analyses of variance for thawing methods of ground pork stored for 16 weeks at -18°C.

Parameter	Mean square	F-value
% moisture (meat)	8.35	7.68 **
Water-holding capacity <sup>a</sup>	0.40	0.08
Color <sup>b</sup> :		
L-value (lightness)	3.32	2.80
a-value (redness)	8.64	8.33**
b-value (yellowness)	1.13	22.20***
%R630-%R580 nm	0.55	0.09
% protein (drip)	0.84	2.50

<sup>1</sup>Mean of 4 replications

<sup>a</sup>Measured by Hamm Press method

<sup>b</sup>Measured by HunterLab D54 reflectance spectrophotometer

\*\* p ≤ 0.01; \*\*\* p ≤ 0.001

Table I-8. Means<sup>1</sup> for a and b-value, %moisture and volume of drip of frozen ground pork thawed by 2 methods.

Parameter	Thawing method	
	Ambient temperature	Refrigerator temperature
a-value	14.33a	15.26b
b-value	5.48a	5.81b
% moisture	58.55a	59.46b
Volume of drip (ml)	7.69	----

<sup>1</sup> Mean of 4 replications

<sup>a,b</sup> Means with the same letter within a row are not different ( $p \geq 0.05$ )

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Part II. Comparison of AOAC methods and near-infrared reflectance spectroscopy for proximate analysis of frozen ground pork packaged in various packaging treatments

### Abstract

Proximate analysis of pork for moisture, protein, and fat was performed using a near-infrared tilting filter spectroscopy scanner (NIRS), and the results were compared with those obtained by the reference AOAC methods. Correlation coefficients ( $r$ ) of 0.717, 0.534, and 0.890 were obtained for moisture, protein, and fat, respectively. Results from the two methods differed ( $p \leq 0.05$ ). The NIRS showed potential for use as a rapid method for proximate analysis in ground pork. However, the correlation coefficients, standard errors of prediction, and biases of this method need to be improved before the NIRS 4250 tilting filter scanner can be used effectively as a rapid method for moisture, protein, and fat determination.

## Introduction

Moisture, protein, and fat determinations are important aspects of many industrial and scientific disciplines. Water is the principal constituent of all raw foods and is also an important structural constituent of processed foods (Karmas, 1980). During meat storage, enzymatic action produces fatty acids which may interact with proteins and denature them, resulting in toughening of meat. Oxidation products of fatty acids also can interact with proteins and may cause insolubilization of proteins by intermolecular cross-linking.

The meat industry has great demand for rapid analytical methods for fresh and processed meats (Olson, 1982; Bjarno, 1982). The major components of interest are moisture, protein, and fat. Reference procedures (AOAC methods) used for compositional analysis are generally time consuming (Pettinatti et al., 1973) and may not be profitably employed during processing by the meat industry (Bjarno, 1981). The reference methods used are: Kjeldahl for crude protein analysis, various solvent extractions methods (e.g. Soxhlet) for fat measurements, and oven drying for moisture determination.

The Kjeldahl method has limitations because in some food products it becomes difficult to convert the organic nitrogen to ammonia, and foods rich in histidine and

tryptophan generally require harsh and long digestion that may result in heat decomposition of ammonia and its subsequent loss (Pomeranz and Meloan, 1982). In the determination of moisture, oven drying is based on weight loss. The sample must be thermally stable and not contain significant quantities of volatile components (Karmas, 1980).

The dairy industry has found infrared technology useful for the rapid determination of lactose, protein, and fat content of milk (Bjarno, 1982). Near-infrared reflectance (NIR) analysis is quick and can be performed by employees with little or no technical background (Rotolo, 1979). The success of this method in the dairy and cereal industries has prompted research of its application and usefulness in the meat industry.

The purpose of this study was to compare AOAC methods and NIR spectroscopy (Pacific Scientific Model 4250) for proximate analysis (protein, fat, moisture determination) in frozen ground pork.

### Literature Review

Near-Infrared reflectance (NIR) spectroscopy is a rapid, effective analytical tool that is used for the determination of moisture and protein in cereal grains (Williams et al., 1978); moisture, protein, and oil content of oilseeds (Hymowitz et al., 1974; Ben-Gera and Norris, 1968); and major constituents in forages (Norris et al., 1976). This simple yet accurate method is readily employed by many processing and quality control laboratories for economical component analyses in a number of different products including processed foods, beverages, textiles, dairy products, tobacco, pharmaceuticals, chemicals, and more recently meat products (Grusby, 1984). A rapid determination of protein, fat, and moisture allows a producer to target purchasing requirements precisely, thereby enhancing subsequent production control. Real-time production parameters can be modified as well as the choice of which batch of meat to use in a particular process (Stark et al., 1986). This affects cost calculations and sale forecasts. It allows for the assessment of variable gross margins to determine which products are producing profits or losses.

The near-infrared region is composed of radiation with wavelengths of 700 - 3000 nm, but wavelengths in the region of 1,000 to 2,600 nm are used in most applications

(Hruschka, 1987). Near-infrared spectroscopy is based on actual number of molecules of individual constituents (Murray and Williams, 1987) and functions in two modes; namely reflectance and transmission . In the reflectance mode, the light beam is directed down towards the sample. Light reflected off the sample is then picked up by four lead sulfide cells equally spaced at 45° above the sample. A ceramic disk is used as a reference in the reflectance mode. In the transmission mode a lead sulfide cell is positioned directly beneath the sample cell and an empty quartz transmission cell is used. The detected signal is fed into a log amplifier, digitized, and sent to a computer (Lanza, 1983). The absorbance data is recorded as log 1/R ( $R$ =reflectance) which varies approximately linearly with the concentration of the absorber (Norris, et al., 1976). A higher (1/R) value means that more radiation has been absorbed ( less reflected) by the sample at that wavelength (Hruschka, 1987; Begley et al., 1984). The data is transformed mathematically to reduce multiplicative effects on reflectance spectra, such as particle size and sample temperature (Lanza, 1983). The complex spectra is easily analyzed utilizing regression methods (Grusby, 1984; Lanza, 1983).

Rotolo (1979) stated that the NIR has been successful as a rapid analytical method because of two major reasons:

1) its ability to analyze a sample through exposure to multiple wavelengths, and 2) the capacity to estimate concentrations of different parameters of interest from a single sample presentation. The accuracy and precision are affected by several factors including packing of the open sample cup (Williams, 1975; Ben-Gera and Norris, 1968; Kruggel et al., 1981; Bjarno, 1981), and especially by sample homogeneity (Williams, 1975; Kruggel et al., 1981).

Standard methods used in meat analysis differ from NIR measurements because the former measures a weight-fraction and the latter measures mole-fractions. Therefore, results will be influenced by density variations. Only when the molecular weight is constant from sample to sample for the component in question will the NIR measurements give a constant accuracy versus the reference (AOAC) methods (Bjarno, 1981). Establishing correlation between chemical and physical methods is very difficult, for example, the Kjeldahl method determines protein nitrogen and not protein by itself; ether extraction determines everything that is extractable by ether, not fat per se; and with regard to oven drying to determine moisture content, moisture plus other volatiles are estimated as moisture (Ono et al., 1984). Hauser and Weber (1978) indicated that NIR can be used to estimate protein, fat, and moisture in cooked bologna-type sausages and that the method was not yet

satisfactory for fresh meat. Apparently, heat generated in the sample drawer caused each consecutive reading to be obtained from a warmer sample, and the temperature of the meat sample can influence the accuracy of prediction equation for estimating fat, protein, and moisture (Kruggel et al., 1981). Furthermore, the high coefficient of variation for moisture and protein in ground meat suggested that infrared reflectance on emulsified meat was superior to that of ground meat. The coefficient of variation for fat in ground and emulsified samples was not different.

While the use of NIR is adequate for moisture and fat analysis, the standard error of prediction for protein needs to be reduced before this technique can be used for the regulatory monitoring of meat (Lanza, 1983; Ono et al., 1984; Bartholomew and Osuala, 1988; Ben-Gera and Norris, 1968; Holden et al., 1986; O'Keeffe, 1987). Some aspects of NIR spectroscopy which require attention are: 1) extent of emulsification of the meat, 2) surface preparation of the meat in the sample cup, 3) effect of storing or freezing meat, 4) variation in protein and fat types and their physical state, 5) effect of pH, and 6) temperature of the meat when estimates of composition are obtained (Kruggel et al., 1981).

It is possible to measure protein, fat, and carbohydrates in about 5 minutes with an accuracy and

precision comparable to that of well-established standard methods; and no expensive or dangerous chemicals are used (Bjarno, 1981). There was no significant difference between the infrared method and the reference methods at the 95% confidence level for determination of protein, fat, and moisture in meat products (Bjarno, 1982; Grusby, 1984; Bartholomew and Osuala, 1988; Nagao et al., 1985; Roberts et al., 1987; Lee, 1985).

#### Materials and Methods

Lean and fat pork was obtained from a commercial source (Flint Hills foods, Alma, Kansas). The lean and fat pork was coarse ground separately using a Hobart grinder (model 4732). The coarse ground pork components were blended to a desired fat content of  $23 \pm 2\%$  by using the Pearson square calculation. After mixing, the coarse ground pork was ground using 1/8" plate. Before packaging, the meat was analyzed for moisture content, protein, and fat using AOAC methods (AOAC, 1984). The ground pork was then packaged in various packaging treatments in 0.5 kg. quantities. The packaging treatments included vacuum packaging in oxygen impermeable CryoVac bags<sup>R</sup> (7" x 12") using SuperVac packer polyvinylchloride (PVC) film which is oxygen permeable, Saran Wrap<sup>R</sup> (Dow Chemical Company), Reynolds heavy duty aluminum foil, and Saran Wrap<sup>R</sup> over wrapped with heavy duty aluminum foil. The samples were then stored at -18°C for 16 weeks. Each of 4 replications was stored in an upright Hotpoint freezer at 1 week intervals. After the designated storage time, samples were thawed at 4°C. After thawing the samples were emulsified using an emulsifier (GL-86, No. 83204) manufactured by Griffith Design Equipment Company in Chicago. The emulsified samples were packaged in vacuum bags and frozen until analyzed.

#### Instrument Calibration

Samples used for calibration were obtained by mixing coarse ground lean and fat pork using the Pearson square calculation to obtain samples with different fat, protein, and moisture values. After mixing, the samples were ground with a Hobart grinder (model 4732) using 1/8" plate, and emulsified with an emulsifier (model GL-86, No.83204). Each of the 42 samples was divided into two subsamples, and packaged in vacuum bags to prevent moisture loss, and frozen until analyzed.

Before analysis, all subsamples were thawed to 4°C and brought to ambient temperature. Each subsample was mixed thoroughly and packed into a quartz cell. The samples were then scanned in duplicates with a Pacific Scientific 4250 NIR scanner, equipped with three tilting filters that provided a continuous scan (291 data points) from 1900 to 2320 nm. The spectral data were stored as Log 1/R, where R is percent reflectance. The data were analysed using software developed by Infrasoft International (Pennsylvania State University). Samples with similar spectral data were grouped, allowing selection of calibration sets with maximum spectral variability. Twenty samples were selected from the 84 samples for use as calibration samples. In addition, ten samples were selected randomly for use as a validation set.

The selected samples were then analysed for protein, moisture, and fat using AOAC methods (Kjeldahl for protein, oven drying for moisture, Soxhlet for fat). The summation of values for each sample had a total greater or equal to 98.5% or less than or equal to 101.5% with a precision of 98% or better, otherwise, the proximate analysis of that sample was not used in the calibration or validation set.

The laboratory values were compared (using the software) with the spectral data for each of the samples in both calibration and validation sets. Multiple step-wise linear regression was then used to select the wavelengths and coefficients in the equations that provided the best statistics: Highest  $R^2$  and lowest standard error of calibration (SEC); and highest  $R^2$  and highest standard error of prediction (SEP). The best equation for each of the constituents (protein, moisture, and fat) was then stored in the systems equation file for use in subsequent routine analysis.

Twenty frozen pork samples packaged in different packaging treatments were thawed and used to test the validity of the developed equations. These samples were analysed in duplicates by both the NIR 4250 scanner and AOAC methods for fat, protein, and moisture.

#### Statistical analysis

Data were analyzed with the Statistical Analysis

System (SAS) as a split-plot treatment structure with four replications (batches of meat). The whole plot treatments were five packaging treatments of the meat and the two methods of analysis (NIR and AOAC) were subplot treatments. All effects in the model were considered fixed. Simple correlation coefficients for fat, protein, and moisture from NIR scanner (model 4250) and AOAC proximate analyses were calculated for comparison.

### Results and Discussion

A summary of the laboratory data used in developing the equations for the Near-infrared reflectance spectroscopy (NIRS) analyses is presented in Table II-1. The data provided maximum spectral variability, which is necessary when developing an equation.

Wavelengths selected by the computer (i.e wavelengths which gave the highest correlation coefficient between the AOAC analysis and the Near-infrared Reflectance spectroscopy (NIRS) for the ground pork calibration) are presented in Table II-2. The calibration correlation coefficients between NIRS and AOAC methods were 0.994, 0.970, and 0.998 for moisture, protein, and fat, respectively. These results are similar to results obtained by Grusby and Testani (1984) which had correlation coefficients of 0.9631, 0.9975, and 0.9922 for fat, moisture, and protein, respectively. Moisture gave a slightly higher standard error of calibration (SEC) when compared to protein and fat. The calibration equation should give a high correlation coefficient ( $r$ ), a low SEC, and a low bias. The SEC measures how well the instrument matches the calibration samples, and bias measures the systematic error between the two average values. Fat gave a higher standard error of prediction for the validation samples (SEP) when compared to moisture and protein. The

SEP was used to measure error between AOAC methods and NIRS analyses for samples used to validate the NIRS.

The number of wavelengths (terms) and mathematical treatment of spectral data are presented in Table II-3. The number of wavelengths selected for each variable was 2. This number depended mostly on the number of calibration samples used, and was determined by the formula,  $n/(10 + 1)$ , where n equals the number of calibration samples. The second derivative of the spectral data was used for fat and moisture, while the first derivative was used for protein. The second derivative mathematical transformation of the spectra was found to give better correlations than first derivative. The second derivative was able to separate overlapping absorption bands and corrected for baseline shift. The first derivative provided the same effect but to a lesser extent.

The comparison between AOAC methods and NIRS values obtained from ground pork packaged in different packaging treatments is presented in Table II-4. The correlation coefficients for AOAC versus NIRS values for moisture, protein, and fat were 0.717, 0.534, and 0.890, respectively; and the correlations were significant ( $p \leq 0.001$  for moisture and fat, and  $p \leq 0.05$  for protein). Correlation coefficients in this study were low in comparison to those obtained by Grusby and Testani (1984),

and Lanza (1983). The lower correlation for protein in this study is in agreement with Kruggel et al.(1981). One possible explanation for this lower value between AOAC and NIRS methods for protein could be that protein absorbs near-infrared radiation much more weakly than does water, and the difference between the high protein and low protein spectras is far less noticeable than those differing in moisture content. Another explanation could be that molecules of the same protein in different samples with different moisture content may become hydrated to different degrees. When this differential hydration occurs, the molecular vibrations may be changed to the extent that wavelengths optimum for determination of protein in some samples may not be optimum for others. The lower correlation coefficients for all variables could be explained by the fact that NIRS is a physical method while the AOAC is both a chemical and physical method. For an example, the AOAC method for measuring protein (Kjeldahl) is not determining protein but nitrogen. The ether extraction determines everything that is extractable by ether and not only fat. With the oven-drying method, we are not really determining only water, but also volatiles. With physical methods, at least in theory, we are determining water, protein, and fat.

The SEP for protein was low when compared with that of

moisture and fat. This probably was due to the increased sample size when scanning samples in the reflectance mode. The accuracy and precision of the instrument are affected by several factors including: packing of the cup (cell), and especially sample homogeneity. The low  $R^2$  for moisture, protein, and fat could be explained partially by the size of the calibration and validation sets. A small calibration set ( $n=30$ ) is susceptible to outliers (that is, compared with the rest of the samples, the lab values do not match the predicted values or the spectras do not look very much like any of the other spectras in the calibration set) and a high bias (2.12 for moisture) indicating that the calibration set might contain one or two fairly large outliers.

There was no significant difference among the various packaging treatments used in this study for protein, fat, and moisture. The two methods (AOAC and NIR) for determining moisture, protein, and fat were significantly different ( $p \leq 0.001$ ) (Table II-5), but related (Table II-4).

### Conclusions

NIR spectroscopy (NIR Model 4250) may be used as a rapid method for proximate analysis (moisture, fat, protein determinations) of ground pork. The correlation coefficients for moisture, fat, and protein could be greatly improved if the instrument is adjusted for high moisture foods in order to increase its sensitivity. The correlation would be improved if a large calibration set is used ( $n \geq 50$ ) as this would allow selection of calibration sets with maximum spectral variability, and would result in a maximum number of terms. While the use of NIR as described in this study resulted in lower correlation coefficients as compared to previous studies (especially for the samples used to test the developed equations), the SEP, SEC, and bias needs to be reduced before the NIR 4250 can be used for the determination of moisture, protein, and fat in ground pork.

Table II-1. Composition of samples analyzed by AOAC methods used for calibration.

Variable	Mean <sup>1</sup>	SD	Range
Moisture	52.42	+ 13.32 -	34.22 - 75.94
Protein	14.71	+ 4.32 -	9.75 - 22.27
Fat	31.92	+ 16.87 -	8.40 - 54.79

<sup>1</sup> Mean of 4 replications

Table II-2. Wavelengths selected and statistical summary of ground pork analysis using NIRS.

Variable	WL1 <sup>a</sup>	WL2 <sup>a</sup>	r	SEC <sup>b</sup> %	SEP(C) <sup>c</sup> %	Bias <sup>d</sup>
Moisture	2194	2167	0.994	1.366	1.564	0.666
Protein	2302	2215	0.970	1.059	1.139	0.045
Fat	2150	2196	0.998	1.174	2.267	-0.054

<sup>a</sup>Wavelengths selected for the calibration equation

<sup>b</sup>Standard error of the calibration samples

<sup>c</sup>Standard error of the prediction samples

<sup>d</sup>Systematic error between the two average values

Table II-3. Wavelengths and mathematical treatment for calibration.

Variable	n	WL <sup>a</sup>	Math <sup>b</sup>
Moisture	30	2	2
Protein	30	2	1
Fat	30	2	2

<sup>a</sup>Number of wavelengths (terms)

<sup>b</sup>Mathematical treatment of spectral data: 1 = first derivative; 2 = second derivative

Table II-4. Statistical comparison between AOAC and NIRS values.

Variable	Mean <sup>1</sup>	SD	n	Bias <sup>c</sup>	SEC <sup>d</sup> %	SEP(C) <sup>e</sup>	r <sup>f</sup>	R2 <sup>g</sup>
Moisture	60.04 <sup>a</sup> 57.92 <sup>b</sup>	1.939 1.166	20 20	2.22 -1.07	2.515 1.384	1.384 0.904	0.717 0.534	0.502 0.275
Protein	16.51 17.58	0.667 1.051	20 20	-1.07 -1.94	2.379 1.406	0.904 0.890	0.534 0.752	
Fat	23.27 25.22	2.608 1.723	20 20	-1.94 -1.723	2.379 1.406	1.406 0.890	0.890 0.752	

<sup>1</sup>Mean for 4 replications

<sup>a</sup>Mean for AOAC values

<sup>b</sup>Mean for near-infrared values

<sup>c</sup>Systematic error between the two average values

<sup>d</sup>Standard error of calibration samples

<sup>e</sup>Standard error of validation/prediction samples

<sup>f</sup>Correlation coefficient of lab. and near-infrared values

<sup>g</sup>Proportion of explained variance

Table II-5. <sup>1</sup>Means for NIRS and AOAC methods for moisture, protein, and fat in ground pork.

Variable	Method of Analysis	
	NIRS	AOAC
Moisture (%)	57.65 a	60.04 b
Protein (%)	15.28 a	16.56 b
Fat (%)	23.27 a	24.92 b

<sup>1</sup>Mean of 4 replications

ab Means with the same letter within a row are not different ( $p \leq 0.05$ )

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### Appendix

Appendix I-1. Simple correlation coefficients among various color parameters of frozen ground pork.

Color Parameter:	a-value	b-value	%630 - %580 nm
L-value	-0.40**	0.28	-0.24
a-value		-0.02	0.79***
b-value			-0.28

\*\* p  $\leq$  0.01; \*\*\* p  $\leq$  0.001

GROUND PORK STUDIES: QUALITY PARAMETERS AS AFFECTED BY  
DIFFERENT PACKAGING TREATMENTS AND THAWING METHODS;  
PROXIMATE ANALYSIS USING NEAR-INFRARED REFLECTANCE  
SPECTROSCOPY

by

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AN ABSTRACT OF A MASTERS'S THESIS

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### Abstract

The effects of various packaging treatments and thawing methods on the quality of frozen ground pork stored for 16 weeks at -18°C were investigated. Color (Hunter L-value, a-value, b-value, %R630 - %R580 nm), water-holding capacity, moisture content, meat and drip protein, and drip losses were measured. Color as measured by %R630 - %R580 nm, a-value, and b-value was affected by both packaging treatments and thawing methods. Thawing at ambient temperature produced drip losses and resulted in samples with lower moisture content than thawing at 4°C. Among the packaging treatments, aluminum foil produced the largest volume of drip when the pork was thawed at ambient temperature. Although statistically not different, among the inexpensive packaging treatments used during the study, aluminum foil offered the best protection against quality loss of frozen ground pork.

Proximate analysis of pork for moisture, protein, and fat was performed using a near-infrared tilting filter spectroscopy scanner (NIRS), and the results were compared with those obtained by the reference AOAC methods. Correlation coefficients ( $r$ ) of 0.717, 0.534, and 0.890 were obtained for moisture, protein, and fat, respectively. Results from the two methods differed ( $p \leq 0.05$ ). The NIRS showed potential for use as a rapid method for proximate

analysis in ground pork. However, the correlation coefficients, standard errors of prediction, and biases of this method need to be improved before the NIRS 4250 tilting filter scanner can be used effectively as a rapid method for moisture, protein, and fat determination.